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A split active site couples cap recognition by Dcp2 to activation

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Abstract

Decapping by Dcp2 is an essential step in 5′–3′ mRNA decay. In yeast, decapping requires an open-to-closed transition in Dcp2, though the link between closure and catalysis remains elusive. Here we show using NMR that cap binds conserved residues on both the catalytic and regulatory domains of Dcp2. Lesions in the cap-binding site on the regulatory domain reduce the catalytic step two orders of magnitude and block formation of the closed state whereas Dcp1 enhances the catalytic step by a factor of ten and promotes closure. We conclude that closure occurs during the rate-limiting catalytic step of decapping, juxtaposing the cap-binding region of each domain to form a composite active site. This work suggests a model for regulation of decapping, where coactivators trigger decapping by stabilizing a labile composite active site.

Degradation of messenger RNA plays a crucial role in animal development¹, cell proliferation^{2,3}, differentiation⁴, stress responses⁵, the adaptive immune system⁶, and transcript quality control⁷. Removal of the 5′-terminal cap structure by the mRNA decapping enzyme Dcp2 is a critical step in numerous 5′–3′ decay pathways including bulk 5′–3′ decay^{8,9}, AU-rich element mediated decay^{10,11}, nonsense-mediated decay (NMD)^{7,12}, miRNA induced decay^{13–15} and 3′ uridylation^{16–19}. In these pathways, decapping is an irreversible step that exposes the 5′ monophosphate of the RNA body for recognition by conserved 5′–3′ exonucleases²⁰. Decapping is thus a highly regulated, committed step that sentences an mRNA to destruction.

Regulation of decapping is achieved by a dense network of protein-protein interactions impinging on the decapping enzyme Dcp2 (refs. ^{7,14,21–25}). Though activation of decapping could be accomplished by targeting the decapping complex to a substrate mRNA, recent

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AUTHOR CONTRIBUTIONS

S.N.F. designed and performed experiments, analyzed data, wrote the manuscript and prepared the figures. B.N.J. performed an experiment, G.A.H. generated Dcp2 mutants, and J.D.G. supervised the project, experimental design, and guided manuscript preparation.

work revealed that a conformational change in yeast Dcp2 is used to achieve a switch like response in activity^{26,27}. Crystallographic studies^{26,28} indicate the conserved N-terminal region of Dcp2 has a bi-lobed architecture containing a regulatory domain, which binds the essential activator Dcp1, and a catalytic domain of the Nudix hydrolase superfamily (Fig. 1a)^{26,29,30}. The co-crystal structure of the *S. pombe* Dcp1-Dcp2 complex (ref. ²⁶) displayed two conformations of Dcp2: one extended and unliganded and the other compact and bound to ATP (Supplementary Fig. 1a). NMR and kinetic studies revealed an RNA binding channel that bisects the regulatory and catalytic domains, suggesting conformational transitions in Dcp2 control an aspect of substrate recognition and catalysis^{26,27}.

Four observations suggest conversion between open and closed states is required for efficient decapping. First, the regulatory domain and Dcp1 together contribute a factor of 1,000 to the catalytic step and ensure correct bond hydrolysis^{27,31} but are far removed from the active site in the open form of the Dcp1-Dcp2 complex (Supplementary Fig. 1a)²⁶. Second, the regulatory domain and Dcp1 are essential for decapping in budding yeast^{9,28}. Third, introduction of prolines in the interdomain linker block closure as detected by small angle X-ray scattering (SAXS) and hinder decapping *in vitro*²⁶. Finally, the proline mutations mimic the effect of a Dcp2 knockout on reporter mRNA half life *in vivo* and cause accumulation of Dcp2 in P-bodies²⁶. Enlarged P-bodies are also a consequence of lesions in a variety of coactivators of decapping, implicating this conformational change in activation of decapping *in vivo*^{14,22,32-34}. These findings support the notion that conformational changes in Dcp2 constitute a critical, regulated step governing mRNA decapping and transcript stability.

An unresolved question is how closure in Dcp2 is linked to efficient cap hydrolysis. Using the crystal structure of the fission yeast Dcp1-Dcp2 complex as a guide (ref. ²⁶), we show that mutation of conserved residues of the regulatory domain retards the catalytic step and blocks closure. Surprisingly, a surface of the regulatory domain specifically interacts with m⁷G whereas the catalytic domain binds multiple nucleotides using different surfaces. We show Dcp1 enhances the catalytic step by a factor of 10 *in vitro* likely by stabilizing the closed conformation²⁶. We propose a composite active site model of decapping, where the regulatory domain makes specific interactions with the cap positioning it for pyrophosphatase chemistry catalyzed by the Nudix domain.

RESULTS

Mutations on the regulatory domain retard the catalytic step

To determine how closure contributes to catalysis we sought to disrupt the closed crystal form (2QKM.pdb, ref. ²⁶) and test for kinetic defects. We selected sites along the 31–52 helix of *S. pombe* Dcp2 involved in the closed-form interdomain interface, mutated them to alanine, and performed kinetic analyses (Fig. 1a,b)^{27,35}. Due to their superior solubility we used decapping proteins from *S. pombe* and cite residue numbers accordingly. Candidate interactions that could stabilize the closed form include a salt bridge between Glu39 and Arg95, an apparent cation- π interaction between Trp43 and Arg167, and a contact between Asp47 and the backbone of Tyr220 (Fig. 1b)²⁶. We narrowed our focus to Trp43 and Asp47 as a previous alanine scanning study indicated mutation of these conserved residues resulted

in the strongest decapping defects observed on a reporter mRNA in yeast²⁸. Alanine substitution at positions 43 and 47 strongly attenuated decapping activity in our single-turnover kinetic assay, consistent with previous *in vitro* endpoint assays (Fig. 1c)²⁸. Mutation at Trp43 or Asp47 reduces the catalytic step (k_{max}) by a factor of 20 or 100 respectively, whereas K_M was nearly unchanged (Fig. 1d and Table 1). Given the location of Trp43 and Asp47 the attenuation of the catalytic step is likely by destabilizing the active species.

Mutations on the regulatory domain block closure

The kinetic analyses above cannot distinguish between defects in conformational changes or chemistry since both are proposed to occur in the catalytic step. As such, we turned to SAXS to directly test the effect of the mutants on conformational changes in Dcp2. It was previously established that a compaction of the Dcp1-Dcp2 complex occurs in the presence of ATP or substrate analogs²⁶. We reproduced these data on a C-terminal deletion construct (1–243) that is more amenable to solution NMR spectroscopy but has the same activity *in vitro* (Fig. 2a and Supplementary Fig. 1b). The distance distribution function $P(r)$ reveals a compaction in the presence of cap-analog m⁷GpppA that is small but reproducible (n=3) (Fig. 2b). In response to nucleotide addition the radius of gyration R_g shrinks from 31.4 ± 1 Å to 28.1 ± 1 Å and the maximum interatomic distance D_{max} is reduced from 135 ± 10 Å to 105 ± 10 Å in the unbound and liganded experiments, respectively. The region of $P(r)$ which changes upon ligand addition (60–100 Å) is similar to the region which changes between the computed $P(r)$ curves from the open and closed crystal forms (Supplementary Fig. 1c–e). Differences between the apo solution scattering and open crystal form (Supplementary Fig. 1c), or the liganded solution scattering and closed crystal form (Supplementary Fig. 1d) likely result from averaging due to a heterogeneous population, potentially including a mixture of the open and closed crystal forms or alternative states.

In contrast to the compaction observed in wild-type Dcp1-Dcp2, SAXS analysis of the Dcp1-Dcp2 W43A mutation shows no change in R_g or the distance distribution function between 60 and 100 Å upon addition of cap analog (Fig. 2c). The D47A mutation did not have a visible effect on the SAXS distance distribution function (data not shown). We therefore conclude that mutation at Trp43 alters the conformational states accessible to Dcp2 and that Asp47 may affect either or both the chemical and conformational steps.

Dcp1 enhances the catalytic step

Previous kinetic studies of *S. cerevisiae* proteins indicated that Dcp1 and the regulatory domain of Dcp2 contribute a factor of 1,000 to the catalytic step of decapping²⁷. Unlike the decapping proteins from *S. cerevisiae*, Dcp1 and Dcp2 from *S. pombe* can be expressed in *E. coli* and purified separately²⁸, allowing us to directly quantify the catalytic enhancement by Dcp1. Dcp1 stimulates the catalytic step (k_{max}) by 10 times with only a small change in K_M (Fig. 1c,d and Table 1). Nucleotide-driven closure of *S. pombe* Dcp2 was only detected by SAXS in the presence of Dcp1, suggesting it may affect the conformational equilibrium that could manifest in this rate enhancement²⁶. Therefore, the enhancement previously measured²⁷ consists of a factor of 10 from Dcp1 and 100 from the regulatory domain.

The ATP-bound and active forms may be distinct

The rationale for mutation at Trp43 is its apparent cation- π interaction with Arg167 in the ATP-bound closed conformation (Fig. 1b)²⁶. However, mutation of the non-conserved residue Arg167 to glutamine (Fig. 1c,d) or alanine has no effect on decapping *in vitro*²⁶. Consistent with the kinetic data, R167Q transitions into a compact state upon addition of m⁷GpppA (Fig. 2d). Differences in the R167Q distance distribution functions (Fig. 2d) compared to wild-type (Fig. 2b) are largely due to a difference in unliganded R167Q versus unliganded wild-type Dcp2. Therefore, Trp43 retards the catalytic step by blocking closure independent of Arg167, and conversely Arg167 is not involved in stabilizing the active conformation of Dcp2. This suggests that the interdomain interface in the active form may be different than that observed in the ATP-bound crystal structure²⁶.

The regulatory domain harbors a binding site for cap

Previously we determined that substrate RNA is bound by the catalytic domain of *S. cerevisiae* Dcp2 and that N7-methyl cap is specifically recognized during the catalytic step (ref. ²⁷). However, it was not possible to address the structural foundation of this effect due to the limited solubility of budding yeast Dcp2 constructs²⁷. Since closure is promoted by nucleotide, we reasoned that formation of a compact, active form was likely driven by direct contacts between the two domains and cap. Therefore, we employed a fragment-based strategy to test the ability of each domain of *S. pombe* Dcp2 to bind m⁷GDP product and a panel of nucleotides by NMR, starting with the regulatory domain (residues 1–94, Fig. 1a). Surprisingly we found that the regulatory domain has an evolutionarily conserved binding site for m⁷GDP (Fig. 3a,b). Strong chemical shift perturbations were detected at both the Trp43 and Asp47 positions and the surrounding 31–52 helix (Fig. 3c,d and Supplementary Fig. 2a). The HSQC titrations were fit well by a two-state binding model that is used throughout to calculate dissociation constants, consistent with the colinearity of the chemical shift perturbations (Fig. 3c,d and Supplementary Fig. 2b). Fitting of HSQC titrations for the Trp43 indole and Asp47 backbone amide yields a K_d for m⁷GDP of roughly 12 mM suggesting a concerted binding process (Supplementary Table 1). Direct binding of cap by the regulatory domain suggests how substrate binding may stimulate closure.

We next tested the specificity of this binding site by titrating different nucleotides against the domain and found that GDP binds weakly to the same site (Fig. 3e,f and Supplementary Fig. 2c) and neither ATP nor other nucleoside diphosphates bound to this site (Supplementary Fig. 2d and data not shown). Quantification of the data shows that the GDP K_d is weaker at ~40 mM (Supplementary Fig. 2e and Supplementary Table 1). Therefore this site is specific for the guanine base and has enhanced affinity for the N7-methylated form. Critically, mutation of Trp43 to alanine blocks m⁷GDP binding, directly implicating Trp43 in the binding process, and suggesting that mutation at Trp43 blocks closure by decoupling the regulatory domain from cap recognition (Supplementary Fig. 2f).

Since m⁷GDP is a product of the decapping reaction, it is possible that its binding to the regulatory domain is different than that of the substrate RNA cap. Therefore, we tested binding of the cap-analog m⁷GpppA and found that it binds to the same surface with nearly identical affinity as m⁷GDP (Supplementary Fig. 3 and Supplementary Table 1). In contrast,

ApppA does not bind the regulatory domain (Supplementary Fig. 4). These data indicate that the addition of adenine, which would correspond to the first transcribed nucleotide of the mRNA, does not affect the recognition of m⁷G by the regulatory domain.

The catalytic domain binds m⁷G and ATP at different sites

If there is a ligand-dependent coupling between the two domains then the catalytic domain should also bind nucleotide. To test this we again used HSQC titration to assess the nucleotide binding ability of the catalytic domain (residues 95–243 of *S. pombe* Dcp2, Fig. 1a). Three regions were perturbed upon addition of nucleotide: the convex dorsal surface (residues 224–243), the ATP-binding pocket in the crystal structure of spDcp1-Dcp2 (residues 116–122, 129, 220–222, ref. ²⁶), and the catalytic helix (residues 136–148). There is a clear difference in the binding profile of m⁷GDP and ATP for these regions (shifts depicted on PDB 2QKM, Fig. 4 and Supplementary Fig. 5). The dorsal surface was perturbed upon addition of either ATP or m⁷GDP (Fig. 4d,f). This region is positively charged and was shown to bind the RNA body nonspecifically, consistent with its lack of specificity for nucleotide²⁷. Similarly, the ATP-binding pocket of the protein is the canonical substrate binding region in Nudix hydrolases²⁹ and binds both nucleotides tested. We directly tested the contribution of the gamma phosphate on ATP by titrating ADP against the catalytic domain and find results similar to ATP but with reduced affinity, consistent with the interaction being driven by electrostatics (Supplementary Fig. 6 and Supplementary Table 1). Importantly, there is excellent agreement between the ATP binding site in the closed crystal state²⁶, the RNA binding channel determined in a previous study²⁷, and the regions detected by NMR chemical shift perturbation in this work.

In contrast to the dorsal surface and the ATP binding pocket, the catalytic helix was selectively perturbed upon addition of m⁷GDP but not ADP or ATP (Fig. 4a,b,e,g and Supplementary Figure 6). Binding of m⁷GDP also caused perturbations around the catalytically essential residue Glu192, pointing at a potential interaction between Glu192 and cap or remodeling of the 161–194 loop that is not detected by NMR in the presence of ATP or ADP (Figs. 1b,4a and Supplementary Figure 5a) The previously determined location of the 5' phosphate of the RNA body places the cap close to the catalytic helix, which is consistent with the perturbations we see upon addition of m⁷GDP. Moreover, we previously demonstrated that residues in the catalytic helix are perturbed by non-hydrolyzable capped RNA but not 5' hydroxyl RNA²⁷. In conclusion, the catalytic helix has a specific binding site for cap while the dorsal surface binds the RNA body.

We selected two probes for quantification: the conserved Nudix motif residue Glu146 on the catalytic helix and Lys235 on the RNA-binding region. ATP binds to the RNA binding helix (Lys235) with a K_d of roughly 5 mM but not detectably to the catalytic helix (Supplementary Fig. 5d and Supplementary Table 1). In contrast, m⁷GDP binds to the catalytic helix (Glu146) with a dissociation constant of 12 mM but only weakly perturbs the RNA binding helix (Supplementary Fig. 5c and Supplementary Table 1). Surprisingly, the affinity of m⁷GDP to the catalytic helix and regulatory domain are nearly identical, hinting at a concerted binding process in the two-domain protein. These results suggest Dcp2 has a

composite active site that forms as a result of closure, with cap specific contacts formed by the regulatory and catalytic domains.

DISCUSSION

In this work we uncovered the role of conformational changes in the catalytic mechanism of Dcp2 and the driving force for conversion into the active form. Dcp1 and the regulatory domain contribute factors of 10 and 100 to the catalytic step, respectively, by modulating conformational states of Dcp2 (Fig. 1). The regulatory domain enhances the catalytic step by specifically binding to m⁷G on a conserved surface surrounding Trp43 and Asp47 (Figs. 1,3 and Supplementary Fig 3). Lesions in the cap binding site block closure and retard the catalytic step by up to two orders of magnitude (Figs. 1 and 2), consistent with the severe decapping defect *in vivo* upon mutation of Trp43 or Asp47²⁸. These findings indicate that conversion to the active form of Dcp2 is driven through specific cap recognition by the regulatory domain and suggest that the active form harbors a composite active site with both domains sandwiching the cap.

Nudix family members contain a conserved catalytic motif with additional insertions or domains conferring substrate specificity^{29,36}. Dcp2 conforms to this paradigm, with the catalytic domain performing chemistry and the regulatory domain providing specificity by accelerating catalysis on N7-methyl capped substrates. Interestingly, the addition of m⁷GDP perturbs residues on the conserved, catalytic Nudix helix whereas ATP and ADP do not (Figs. 4 and 5a). The regulatory domain may contribute to catalysis by positioning substrate as it is required to hydrolyze the correct phosphate bond³¹. High-resolution structural studies of Dcp2 in complex with substrate will be required to confirm this prediction.

The cap-bound conformation is different than the ATP-bound closed form for four reasons. First, the cap-binding residues Trp43 and Asp47 of the regulatory domain are buried in the closed structure and do not contact ATP (Fig. 1a,b)²⁶. Second, the regulatory domain and catalytic helix both bind m⁷GDP by NMR but are separated by ~20 Å in the closed crystal structure (Fig. 5a)²⁶. Third, Trp43 forms an apparent cation- π interaction with Arg167 of the catalytic domain but mutating Arg167 to glutamine or alanine does not affect closure or decapping *in vitro* (Fig. 1b–d)²⁶. Fourth, in the closed, ATP-bound form of Dcp1-Dcp2, non-conserved residues 244–266 of the catalytic domain fold into a helix that interacts with the regulatory domain, yet deletion of these residues has no effect on decapping or closure *in vitro* (Supplementary Fig. 1b)^{26,28}. It is unclear whether the ATP-bound form represents an on-or off-pathway state, but it is accessible in solution since ATP causes closure²⁶. Dcp2 may assume different conformations from specific or non-specific interactions with substrate, as described for other nucleic acid binding proteins^{37,38}. Given the composite active site suggested here and the previous crystal structure with open and closed conformations(ref. ²⁶), we conclude that the native energy landscape of Dcp2 is rugged with multiple accessible states depending on the ligand.

Activation of decapping by closure was postulated to be a late step in 5'-3' decay that is promoted by coactivators^{26,33}. Dcp2 with proline hinge mutants blocking closure still localizes to P-bodies but decay of messages is blocked²⁶. Large P-bodies containing Dcp2

and decay substrates are a hallmark of stalled decay complexes or overwhelmed decay machinery and are observed in coactivator mutants^{22,32,34}. The proline hinge mutants also cause a dramatic defect in bulk mRNA decay both *in vitro* and *in vivo*²⁶. The conformational change detailed here is likely part of this late, regulated step since mutation at Trp43 mimics the defect of the proline hinge mutants on closure *in vitro* and *in vivo*²⁸. Therefore, closure and formation of the composite active site may couple decapping to stimulation by coactivators.

Our results are consistent with a model of decapping by Dcp2 where the catalytic step consists of at least two substeps beginning with isomerization to the active form (Fig. 5b) followed by the chemical step (Fig. 5c and Supplementary Note)³⁹. In the open form the active site is incomplete, resulting in inefficient decapping (Fig. 5b and Supplementary Figure 1a). Cap recognition by both domains induces closure and forms a composite active site, allowing for efficient decapping (Fig. 5c). Previous experiments revealed that 5'-monophosphate product RNA and non-hydrolyzable substrate RNA bind with the same affinity, suggesting cap recognition and closure occur after binding²⁷. Dcp1 may accelerate the catalytic step by stabilizing the composite active site or enhancing the rate of closure. Like Dcp1, coactivators of decapping such as the Edc proteins in yeast^{40,41}, the NMD factors Upf2 and Upf3 (ref. ³³), the heptameric Lsm1–7 complex³², Pat1³² and others may stimulate decapping by altering this conformational equilibrium. This is similar to regulation of DEAD-box helicases by cofactors that couple conformational changes to ATP hydrolysis using a bipartite active site^{42,43}. The scavenger decapping enzyme DcpS also utilizes a composite active site to decap 3'-5' exosomal decay products but is different in fold, chemistry, and regulation^{44,45}. For Dcp2, coactivators play a critical role in regulation and the new understanding of the active form presented here motivates further mechanistic studies to determine how regulation is achieved in different 5'-3' mRNA decay pathways.

METHODS

Protein purification, mutagenesis, and decapping complex formation

S. pombe Dcp2 (spDcp2) 1–243 and *S. pombe* Dcp1 1–127 were PCR amplified from *S. pombe* cDNA and cloned into p-His-GB1-parallel⁴⁷. The catalytic domain(95–243) was PCR amplified from spDcp2 1–243 and cloned into p-His-GB1-parallel, while the regulatory domain (1–94) was PCR amplified from spDcp2 1–243 and cloned into a vector derived from pet28a consisting of a His-tag, Tobacco Etch Virus (TEV) protease site and a multiple cloning site (His-TEV-Dcp2–1–94). All proteins were expressed independently in *E. coli*. Cells were lysed by sonication, clarified by centrifugation at 16,000g then overexpressed proteins were purified using Ni-NTA affinity chromatography. Elutions were digested with TEV protease and then subjected to a second Ni-affinity column to remove the His-GB1 tag. The second Ni-purification was not used in purification of Dcp2 1–94 because the His tag is easily resolved by gel filtration. The Dcp1-Dcp2 complex was formed following TEV protease cleavage by incubation of 1.1:1 Dcp1:Dcp2 molar ratio for four hours in 4 °C prior to gel filtration. All mutants were made using whole plasmid PCR with mutagenic divergent primers; sequences of the Dcp2 ORF were confirmed by dideoxy sequencing.

NMR Spectroscopy

Proteins were purified as above and subjected to gel filtration chromatography using a Superdex G75 column in 200 mM NaCl, 100 mM Na₂SO₄, 5 mM DTT, 21.1 mM NaH₂PO₄, and 28.8 mM Na₂HPO₄ pH 7.0 for storage and backbone resonance assignment. Prior to nucleotide titrations, proteins were exchanged into 150 mM NaCl, 2 mM MgCl₂, 5 mM DTT and 50 mM HEPES pH 7.0 using a desalting column (BioRad). Titrations on the regulatory domain were done at 100 μ M protein in a volume of 500 μ L by simultaneous addition of equimolar nucleotide and magnesium. Titrations on the catalytic domain were identical except done in the absence of magnesium. All titration experiments were conducted on a Bruker Avance 800 MHz spectrometer outfitted with a cryogenic probe. Titration data were only fitted if the change in chemical shift between apo and 30 mM nucleotide for a given residue was greater than the mean plus one standard deviation. Assignments were obtained from standard triple-resonance experiments⁴⁸ (HNCA, HNCOCA, HNCO, HNCACO, HNCACB, and HNCOCACB) on a Varian 600 MHz spectrometer with additional verification from a ¹⁵N-dispersed NOESY on a Bruker Avance 800MHz spectrometer. All assignment experiments were performed using cryogenic probes at 500 μ M protein. Assignments were obtained for 88 of 94 residues in Dcp2 1–94 (the regulatory domain) and for 104 of 148 residues in Dcp2 95–243 (the catalytic domain). In Dcp2 95–243 the majority (30 of 44) of the unassigned residues were in a loop (from 194–224) that is not visible in the nitrogen HSQC.

Small-angle X-ray scattering

Samples were purified as described and subjected to gel filtration chromatography using a Superdex G75 column in 150 mM NaCl, 2 mM DTT and 50 mM HEPES pH 7.0. All SAXS experiments were done on stoichiometric Dcp1-Dcp2 complex with mutations in Dcp2 as indicated. Raw data was processed via scripts provided by ALS Beamline 12.3.1 scientists⁴⁹ and buffer was subtracted. In some cases buffer subtraction was done manually by reweighting the normalization of the protein scattering to the buffer scattering because there were small differences in nucleotide concentration between protein and buffer blank. This manual subtraction did not affect the distance distribution function and was used to achieve an intensity curve that asymptotes to zero at high values of momentum transfer. In no case was this correction larger than 5%. Following raw data processing, distance distribution functions were computed using GNOM⁵⁰. D_{max} was explored between 80 and 150 Å in increments of 5 Å and the smallest value that led to a monotonic decrease towards $P(D_{max})=0$ was selected. $P(D_{max})=0$ was then enforced via GNOM. Notably the D_{max} parameter is simply an integration limit for the indirect Fourier transform method of calculating the distance distribution function and is not necessarily a measure of the maximum particle dimension since it is highly sensitive to aggregation^{50–52}.

Decapping assays

Proteins were purified as described and subjected to gel filtration chromatography using a Superdex G75 column in buffer containing 100 mM NaCl, 5% (v/v) glycerol, 5 mM DTT and 50 mM HEPES pH 7.5. Proteins were prepared for kinetics as described³⁵. Rates were measured at a series of enzyme concentrations on a 29-mer substrate²⁷ in an iceblock at ~0.1

°C. Rates for each enzyme concentration were calculated by dividing the initial rate during the linear phase of the reaction by the endpoint of 0.85. Each assay was repeated at least twice and the rate for each enzyme concentration averaged between experiments. These average rates were then plotted versus concentration with error bars representing the standard error of the mean between independent experiments. Data were fitted to extract K_M and k_{max} as described³⁵.

Solvent accessibility calculations

The solvent-accessible surface area⁵³ (SASA) for the regulatory domain was calculated using the program area imol within the CCP4 suite⁵⁴. The open and closed SASA values were calculated using the open or closed crystal structures, respectively (PDB entry 2QKM, ref. ²⁶). The SASA for each residue was normalized to 75% of the maximum value calculated from a Gly-X-Gly tripeptide⁵⁵. The factor of 75% was introduced because the helical 31–52 region is less exposed than a Gly-X-Gly tripeptide and the largest observed SASA was close to 75% of the Gly-X-Gly value (residue 33).

Multiple sequence alignments

Sequences for Dcp2 from ten organisms were retrieved from the NCBI database, aligned using MUSCLE⁵⁶, and visualized using Jalview⁵⁷. Shown in Figure 1 is a fragment of an alignment that was carried out using default MUSCLE parameters on the entire sequence of Dcp2. The species used for the alignment were *S. pombe*, *S. cerevisiae*, *C. albicans*, *A. thaliana*, *C. elegans*, *D. melanogaster*, *X. laevis*, *D. rerio*, *M. musculus* and *H. sapiens*, with 8 of these shown.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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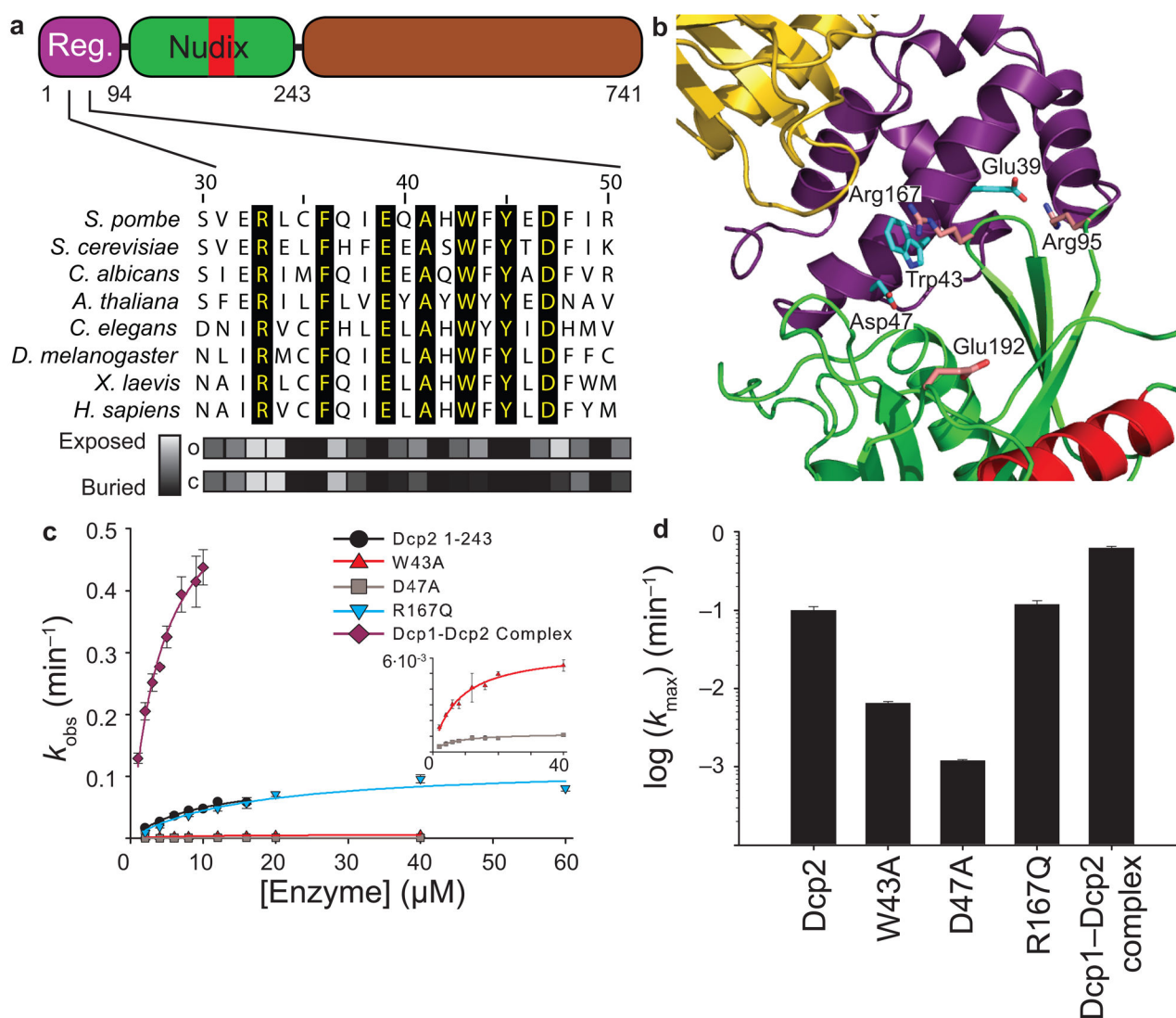
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**Figure 1.**

Mutants along the interdomain interface in the closed crystal form attenuate the catalytic step of decapping. **(a)** Dcp2 consists of an N-terminal regulatory domain (1–94, purple), a catalytic domain of the Nudix superfamily (95–243, green with the Nudix motif in red), and a large, nonconserved C-terminal region of unknown function (244–741, brown). A sequence alignment is provided of residues 30–50 of Dcp2 with absolutely conserved residues highlighted in yellow. For residues 30–50 the solvent accessible surface area (SASA) is shown on a relative scale from buried to exposed in both the open (o) and closed (c) crystal forms, for details see Methods. **(b)** The interface between the regulatory domain (purple) and the catalytic domain (green) in the closed, ATP-bound crystal structure is shown with sites of mutation highlighted (PDB entry 2QKM, ref. ²⁶). Dcp1 is in gold and the catalytic Nudix helix of Dcp2 is in red. **(c)** Observed rate constants for decapping assays of various constructs as marked. Solid lines are fit to a rate equation appropriate for single-turnover kinetics, where enzyme is in excess of substrate (see Supplementary Note)³⁵. Error bars are standard error in the mean for the rate measured in two or more independent

experiments. **(d)** The rate of the catalytic step (k_{max}) is plotted on a log scale for selected interface mutants; error is s.e.m.

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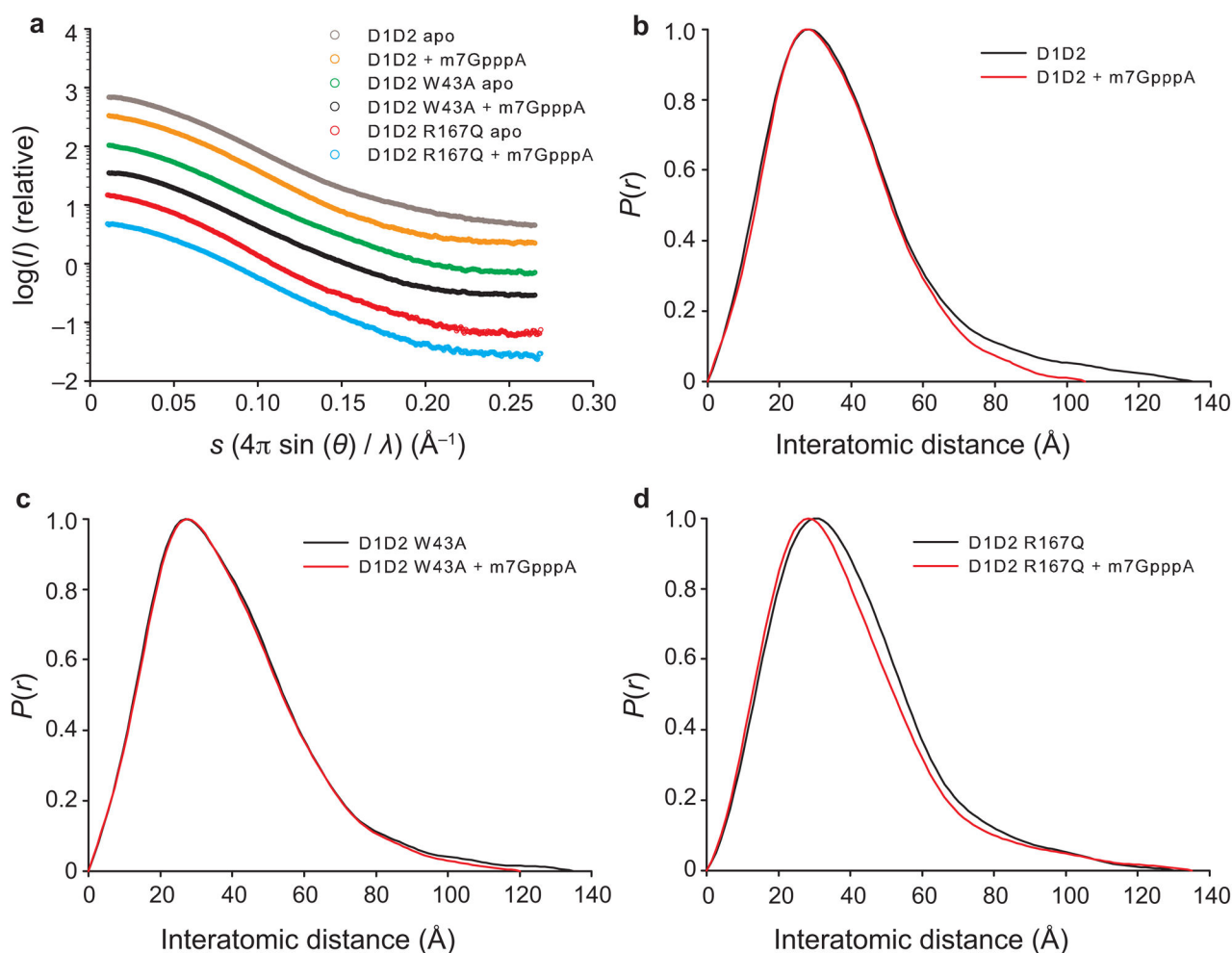


Figure 2.

Some interface mutants block conversion to the closed form measured by SAXS. **(a)** Experimental SAXS curves for the wild-type Dcp1-Dcp2 complex (D1D2) and mutants. Shown is the momentum transfer s , where 2θ is the scattering angle and λ is the X-ray wavelength. Scattering curves are artificially displaced along the ordinate for clarity. **(b)** Plotted is the distance distribution function $P(r)$ vs interatomic separation (r) for the wild-type decapping complex. Unliganded Dcp1-Dcp2 complex (D1D2) is in black and the curve in the presence of 10 mM m7GpppA is in red. A compaction is evident by the reduced probability between 60 \AA and 100 \AA in nucleotide bound (red) versus apo (black). **(c)** The observed compaction is blocked upon W43A mutation of Dcp2. **(d)** The R167Q mutation of Dcp2 still closes upon addition of nucleotide.

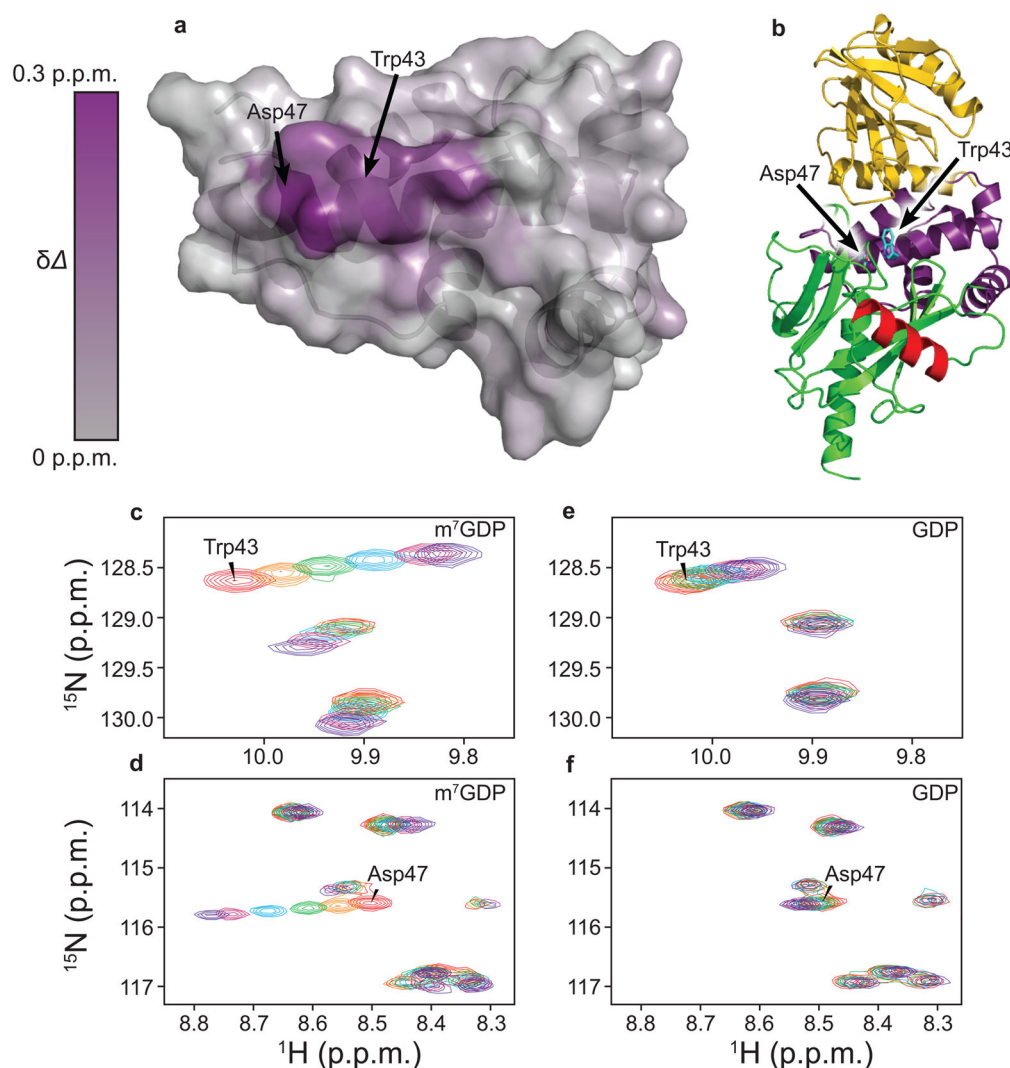


Figure 3.

The regulatory domain has a specific binding site for m^7G . **(a)** The regulatory domain colored by chemical shift perturbation upon addition of 30 mM m^7GDP . Sites of mutation and quantitative analysis Trp43 and Asp47 are indicated. **(b)** A view of the Dcp1-Dcp2 complex aligned with the isolated regulatory domain (PDB entry 2QKM, ref. ²⁶). Colors as in figure 1b. **(c,d)** HSQC spectral overlay of the Trp43 indole region **(c)** or the Asp47 backbone **(d)** while being titrated by m^7GDP . Concentrations of nucleotide shown are 0mM (red), 2.5 mM (orange), 5 mM (green), 10 mM (cyan), 20 mM (pink), and 30 mM (purple). **(e,f)** HSQC spectral overlay of the Trp43 indole region **(e)** or the Asp47 backbone **(f)** over the same concentration range of GDP.

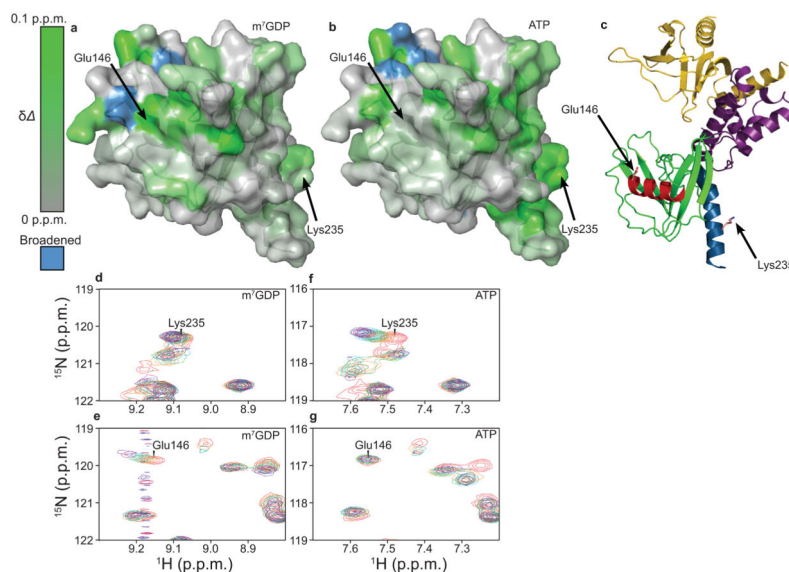
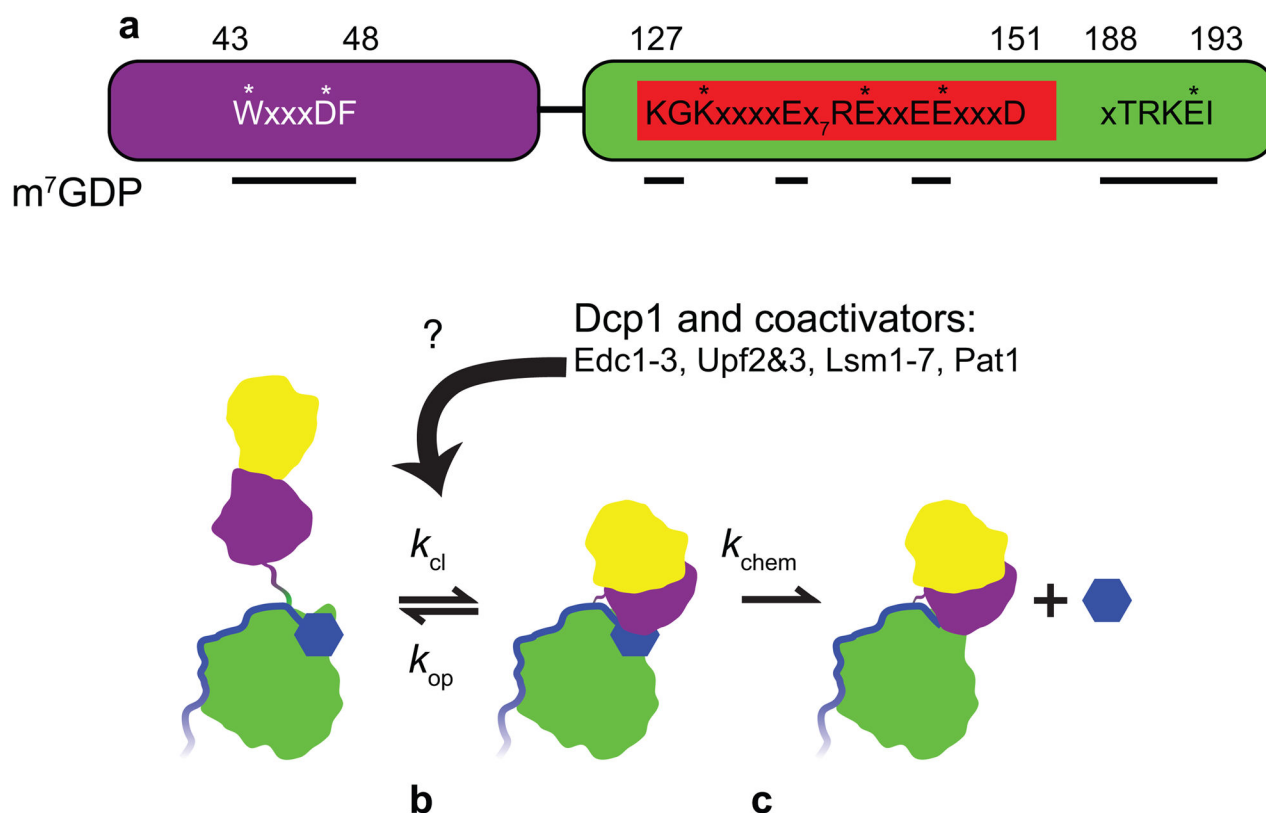


Figure 4.

The catalytic domain binds m^7 GDP on the catalytic helix and binds a variety of nucleotides along the RNA binding region. **(a)** The catalytic domain colored by chemical shift perturbation upon addition of 30 mM m^7 GDP. Amide groups of Glu146 and Lys235 are used as representatives for binding to the catalytic helix and the RNA binding region, respectively. Blue indicates resonances that were broadened beyond detection upon addition of nucleotide. **(b)** The catalytic domain colored by chemical shift perturbation upon addition of 30 mM ATP on the same scale as **(a)**. **(c)** A view of the Dcp1-Dcp2 complex aligned with the isolated catalytic domain (PDB entry 2QKM, ref. ²⁶). Colors as in Figure 1b, with the RNA-binding region highlighted in blue. **(d, e)** HSQC spectral overlay of Lys235 **(d)** or Glu146 **(e)** while being titrated by m^7 GDP. Colors are the same as in Figure 3. The noise in the m^7 GDP Glu146 region results from incomplete suppression of uncoupled magnetization of nucleotide due to the phase cycling used in the FHSQC sequence and did not affect interpretation of the data or the fit.⁴⁶ **(f, g)** HSQC spectral overlay of Lys235 **(f)** or Glu146 **(g)** being titrated with ATP.

**Figure 5.**

Decapping by Dcp2 proceeds by formation of a composite active site involving both the regulatory and catalytic domains. **(a)** Regions on the regulatory and catalytic domains that are perturbed by $m^7\text{GDP}$ or $m^7\text{GpppA}$ cap analog but not other nucleotides are indicated with a bar. Residue boundaries of the regions are indicated above the diagram. Conserved residues are shown in capital letters and those that have strong effects on decapping *in vivo* when mutated are marked with a *. Highlighted in red is the conserved Nudix motif, consisting of $GX_5EX_7REUXEEXGU$ with X any residue and U is I, L, or V. **(b)** Isomerization into the active form requires direct interactions of both domains with cap. Colors as in Figure 1b with RNA indicated in blue with a hexagon cap. **(c)** Once the active state is formed decapping proceeds efficiently using a composite active site with cap sandwiched between the two domains.

Table 1

Kinetic rate constants for Dcp2 in vitro.

| Protein | K_M (μM) ^I | k_{max} (min^{-1}) ^I | k_{max}/K_M ($\text{min}^{-1}/\mu\text{M}$) | k_{max}/K_M (mut/WT) |
|-------------------|--------------------------------------|---|--|-------------------------------|
| WT Dcp2 1–243 | 10.1 ± 2.30 | $9.98 \times 10^{-2} \pm 1.16 \times 10^{-2}$ | 9.88×10^{-3} | 1 |
| W43A | 7.42 ± 0.938 | $6.52 \times 10^{-3} \pm 3.00 \times 10^{-4}$ | 8.78×10^{-4} | 0.089 |
| D47A | 5.43 ± 0.737 | $1.19 \times 10^{-3} \pm 5.20 \times 10^{-5}$ | 2.19×10^{-4} | 0.022 |
| R167Q | 16.2 ± 4.75 | $1.18 \times 10^{-1} \pm 1.41 \times 10^{-2}$ | 7.28×10^{-3} | 0.74 |
| Dcp2 1–243 + Dcp1 | 4.33 ± 0.487 | $6.19 \times 10^{-1} \pm 3.01 \times 10^{-2}$ | 1.43×10^{-1} | 14 |

^I Error is s.e.m.